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In Memoriam: Maria Rosa Miracle (1945-2017)

Dear Colleague,

It is with great regret we must report that our friend and colleague Dr. Maria Rosa Miracle sadly passed away on 21st May 2017, after an illness of over one year. May she rest in peace.

Maria Rosa Miracle was born on 2nd June 1945 in Barcelona. She studied at the University of Barcelona where she was awarded both her BSc degree (honours) and, in 1974, her PhD (cum laude).

As a former PhD student of Dr. Ramon Margalef she conducted field work at Lake Banyoles (Girona) studying along several years the seasonal succession, distribution and patchiness of zooplankton, supervised by Dr. Margalef, then completing her PhD dissertation after a two years research stay at the University of California, Davis, with Dr. Charles Goldman as supervisor of the stay.

Upon returning to Spain she spent several years, until 1979, at the University of Barcelona as Associate Professor, developing mainly two projects financed by Research Scholar Government Public Works: the “Study on Pyrenean Lakes” and a ”Study on the Spanish Mediterranean coastal lagoons”. In 1979 she joined the University of Valencia as Senior Professor of Ecology, setting up the Limnology Research Team there, and progressing to Chair on Ecology in 1981, following her academic activity until her retirement on the first of September 2015. Thereafter, she continued as Emeritus Professor at the University of Valencia.

During more than forty years, Maria Rosa Miracle was an unflagging worker with her activity completely devoted to research and academic purposes. She served as the Head of the Ecology Department at the University of Valencia between 1981-1987 and 2003-2010, as the President of the Academy of Environmental Biology, India, from 1987 to 1990, and as President of the Iberian Limnological Association from 1994 to 2002. She supervised more than 20 PhD students most of which are nowadays university professors, scientists, or environmental managers on public or private entities, as well as more than 20 Master Theses. She was also Principal Investigator or participant in over 20 Spanish-funded research projects, over 10 European or International Projects, and 18 technical studies and reports. She authored or co-authored more than 200 indexed scientific published papers, 21 book-chapters or books, and organized 16 International Congresses or Workshops. She also authored over 250 congress communications. This is just a numerical summary of forty years of professional activities.

In addition to the study many of Spanish lakes (mainly karst lakes), wetlands and lagoons, she also visited and studied aquatic ecosystems in many other countries, collaborating with some of the most reputed limnologists around the world. Among others, she shared research with Henri Dumont, performing a limnological expedition to the Argelian and Tunisian Sahara in 1976 and a study of primary production in marginal lakes associated with River Niger in 1993; with Charles King, on rotifer population dynamics and experimental laboratory studies on zooplankton survival in 1979; and with Bland Finlay at the Windermere Laboratory on a study of microaerophilic and anoxic environments in 1983, and electron microscopy studies of ciliates and bacteria at the oxic-anoxic interfaces in 1992. She also conducted research with Peter Tyler on the study of interfaces and chemoclines in the stratified lakes of Gordon river in Tasmania in 1987; with Wayne Wurtsbaugh, studying the eutrophication of a basin in the Great Salt Lake in 2002; with Keve Kiss, of the Hungarian Academy of Sciences, developing an Spain-Hungary integrated project for plankton studies from 2005 to 2007 and, most recently, with Victor Alekseev, of the Russian Academy of Sciences, jointly studying the cyclopoid groups of confusing identification by comparison with specimens from type locality in 2011, as well as conducting a study of microcrustaceans at the shoreline of the Lake Baikal and wetlands near Irkutsk in 2012.

Similarly, reputed senior scientists have visited Maria Rosa's laboratory to study the Albufera de Valencia and other shallow lakes in the Mediterranean coast, including most of the above mentioned but also Brian Moss and S.S.S. Sarma. Another limnological area of her interest, the stratified solution lakes of Arcas and the karst area at Cañada del Hoyo with the internationally well-known meromictic Lake La Cruz (both systems in Cuenca, Spain), was visited by her with other senior scientists such as Dr. Jakob Zopfi and Prof. Raymond Cox, to mention some additional to the already mentioned collaborators.

All her scientific and academic achievements were only possible through her lively and passionate attitude towards the challenges of the new, the uncommon and the unknown, with a desire and disposition to increase the knowledge of extreme or strange environments wherever they might be encountered, seeking those inland waters, lakes, wetlands in Europe, America or Africa even getting to the barely reachable Saharan or fluvio-marginal lacustrine waters at the Niger Delta.

In addition to her achievements in Academia, the most remarkable feature about her character is that she was a hard worker and a very good and gentle person. Maria Rosa was all her life a great supporter of Spanish Limnology and will be missed by her colleagues and all who has known her.

Eduardo Vicente and Antonio Camacho

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On the possible use of Mongolian branchiopods and copepods to establish reference conditions for ecological quality assessment of lacustrine water bodies in Spain

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ABSTRACT

On the possible use of Mongolian branchiopods and copepods to establish reference conditions for ecological quality assessment of lacustrine water bodies in Spain

A comparison of assemblages of Branchiopoda and Copepoda living in lakes and wetlands in two very distant territories in the Palaearctic Region, the Iberian Peninsula and Mongolia, is presented. Both territories have significant similarities in the hydromorphological and physicochemical characteristics of their water bodies and in the structure and composition of their fauna of branchiopods and copepods. Some species occur in both areas, others are taxonomically closely related, and others behave as geographic vicariants. Most of these microcrustaceans are used as indicators of ecological quality of standing water bodies in industrialized European countries, where reference sites are frequently difficult to find. Taking into account the undisturbed conditions of Mongolian water bodies, the alternative of using their branchiopods and copepods, after setting the proper, statistically-based equivalence between Mongolian and Iberian species, as references to assess ecological status in Spain is discussed.

Key words: Branchiopoda, Copepoda, Spain, Mongolia, lake ecological status assessment, reference conditions.

RESUMEN

Sobre el posible uso de Branquiópodos y Copépodos de Mongolia en el establecimiento de condiciones de referencia para el análisis de la calidad ecológica de las masas de agua lacustre en España

Se presenta una comparación de grupos de Branchiopoda y Copepoda de lagos y humedales en dos territorios muy distantes de la región paleártica: la península ibérica y Mongolia. Los dos territorios tienen similitudes significativas en las características hidromorfológicas y fisicoquímicas de sus masas de agua, y también en la estructura y composición de su fauna de branquiópodos y copépodos. Algunas especies coinciden, otras son muy próximas taxonómicamente y otras se comportan como vicarias geográficas. Muchos de estos microcrustáceos se utilizan como indicadores de calidad ecológica en las masas de agua léntica de los países europeos industrializados, donde las condiciones de referencia son frecuentemente difíciles de encontrar. Teniendo en cuenta la situación inalterada de las masas de agua de Mongolia, se discute la alternativa de utilizar sus copépodos y branquiópodos, tras el necesario análisis de las equivalencias entre ellos y los de la península ibérica, como referencia para determinar el estado ecológico de los lagos en España.

Palabras clave: *Branchiopoda, Copepoda, España, Mongolia, evaluación del estado ecológico en lagos, condiciones de referencia.*

INTRODUCTION

Maintaining the "good ecological status" of water bodies is the main objective of the Water Framework Directive (WFD) (200/60/CE) in all European countries. This Directive was transposed into Spanish law at the end of 2003. Subsequently, the *Instrucción de Planificación Hidrológica* (hereafter IPH) (Ministerio de Medio Ambiente y Medio Rural y Marino, 2008) was implemented, with its objective being to establish the technical criteria to systematically and homogeneously develop River Basin Management Plans in Spain. Concerning lakes, the IPH defines the systems and protocols for the ecological status assessment, including the definition and exploitation of the biological control networks, and establishes indicators, indexes and reference conditions. The latter are those presented in water bodies of the same category and type as those intended for assessment under pristine conditions or barely altered by anthropic activities.

Several quality elements are taken into account in the ecological status assessment of lakes: biological (phytoplankton, phytobenthos, macrophytes, benthic invertebrates and fishes), physicochemical and hydromorphological. As this paper is concerned with branchiopods and copepods, it is necessary to refer to the IBCAEL (*Índice Biótico de Calidad Ecológica en Lagos*) published by the Ministerio de Agricultura, Alimentación y Medio Ambiente of the Spanish Government in 2013, whose application is mandatory in the exploitation of the official networks for lake ecological status assessment in compliance with the WFD. The IBCAEL is an adaptation of the QAELS (*Qualitat de l'Aigua en Ecosistemes Lenítics Soms*) index for the whole Spanish territory designed to assess the ecological status of shallow water bodies in northeast Spain (Catalonia) (Boix *et al*., 2005). The IBCAEL is calculated as the combination of two subindexes: ABCO (abundance of benthic branchiopods, copepods, and ostracods) and the RIC (insect and crustacean richness). The organisms included in both the ABCO and RIC are good indicators because they are very sensitive to disturbances and changes in their habitats (Stemberger & Lazorchak, 1994; Hofmann, 1996; Moreno-Amich *et al*., 1999; Johansson *et al*., 2005) and spend their entire life cycles, which are relatively long, in the water. Moreover, because some of these microcrustaceans, mainly cladocerans and ostracods, leave remains in the sediment that have been largely used in palaeolimnological studies (Kattel & Sirocko, 2011; Horne *et al*., 2012), they are an appropriate tool for searching for the existing species assemblages prior to the existence of anthropic pressures, that is, reference conditions.

Branchiopods and copepods of continental standing waters are well known in Spain (Alonso, 1996; 1998); however, the information regarding these taxa in Mongolia is scattered and incomplete (Sars, 1903; Brtek *et al*., 1984; Naganawa & Zagas, 2002; Flossner *et al*., 2005; Penkova *et al*., 2005; Pomazkova & Sheveleva, 2006; Alonso & Naganawa, 2008; Sinev *et al*., 2009; Alonso, 2010; Alonso & Ventura, 2013). In fact, the most comprehensive inventory for this country is currently being performed by the author of this paper, with some of the taxa mentioned below only partially reported in the abovementioned publications.

Reference conditions in Spain are relatively easy to find in high mountain lakes; however, they are practically absent in lowlands due to the ancient occupation of the totality of the territory by agricultural activities and human settlements. In particular, the lakes in the steppes, which were the jewel of the Spanish lacustrine heritage in the past and are almost entirely unique in Western Europe, have disappeared or have been significantly altered. In Mongolia, steppes are widespread and host a huge number of lakes in pristine conditions. Although the Mongolian and Iberian steppes are bioclimatically different, their lakes are similar. This and the presence of even the same or vicarious species and similar species assemblages in both distant territories could allow us to use ABCO values obtained from undisturbed Mongolian lakes as reference conditions for the Spanish lakes.

In this paper, Spanish and Mongolian lakes and lagoons and their branchiopod and copepod assemblages are compared. The convenience of using the conditions of Mongolian lakes as a reference for Spanish lakes is discussed.

MATERIALS AND METHODS

Samples were collected during an extensive survey performed throughout the Mongolian territory, considering all of its natural zones (alpine belts, taiga, forest-steppe, steppe, semidesert and desert zones) between 2005 and 2013. In total, 878 lakes were sampled. Abbreviated descriptions of all the lakes and photos can be seen at http://geodata.es/mongolian_lakes. The surveys were conducted in September-October, after the rainy season and before the freezing of the lake surface; during this period, the crustacean communities reach their peak of maturity, and males and gamogenetic females appear in the cladoceran populations. Samples were obtained from representative habitats in each lake (littoral ar-

Table 1. Correspondence between the IPH and IBCAEL typologies of lakes and lagoons in Spain and the typologies defined for Mongolian lakes in this paper. *Correspondencia entre las tipologías IPH e IBCAEL de lagos y lagunas en España y la tipología definida para los lagos de Mongolia en este trabajo.*

<table>
<tr><th>Typology IPH</th><th>Description</th><th>Typology IBCAEL</th><th>Typology MONGOLIA</th></tr>
<tr><td>1</td><td>NORTHERN HIGH MOUNTAIN. DEEP. ACIDIC WATERS</td><td rowspan="5">1</td><td rowspan="12">A</td></tr>
<tr><td>2</td><td>NORTHERN HIGH MOUNTAIN. DEEP. ALKALINE WATERS</td></tr>
<tr><td>3</td><td>NORTHERN HIGH MOUNTAIN. SHALLOW. ACIDIC WATERS</td></tr>
<tr><td>4</td><td>NORTHERN HIGH MOUNTAIN. SHALLOW. ALKALINE WATERS</td></tr>
<tr><td>5</td><td>NORTHERN HIGH MOUNTAIN. DEEP. ALKALINE WATERS</td></tr>
<tr><td>6</td><td>MEDIUM MOUNTAIN. DEEP. ACIDIC WATERS</td><td rowspan="3">2</td></tr>
<tr><td>7</td><td>MEDIUM MOUNTAIN. DEEP. ALKALINE WATERS</td></tr>
<tr><td>8</td><td>MEDIUM MOUNTAIN.SHALLOW. ALKALINE WATERS</td></tr>
<tr><td>9</td><td>SOUTHERN HIGH MOUNTAIN</td><td>1</td></tr>
<tr><td>10</td><td>KARSTIC, CALCAREOUS, PERMANENT, HYPOGENIC</td><td rowspan="3">2</td></tr>
<tr><td>11</td><td>KARSTIC, CALCAREOUS, PERMANENT, UPWELLING</td></tr>
<tr><td>12</td><td>KARSTIC, CALCAREOUS, PERMANENT, TRAVERTINIC</td></tr>
<tr><td>13</td><td>KARSTIC, CALCAREOUS, TEMPORARY</td><td>7</td><td>B</td></tr>
<tr><td>14</td><td>KARSTIC, EVAPORITES, HYPOGENIC OR MIXT, LARGE</td><td rowspan="2">3</td><td>A</td></tr>
<tr><td>15</td><td>KARSTIC, EVAPORITES, HYPOGENIC OR MIXT, SMALL</td><td rowspan="2">B</td></tr>
<tr><td>16</td><td>INTERIOR IN SEDIMENTATION BASIN (ISB), LOW MINERALIZATION,PERMANENT</td><td>4</td></tr>
<tr><td>17</td><td>ISB, LOW MINERALIZATION, TEMPORARY</td><td>7</td><td>B-D</td></tr>
<tr><td>18</td><td>ISB, MEDIUM MINERALIZATION, PERMANENT</td><td>4</td><td>C</td></tr>
<tr><td>19</td><td>ISB, MEDIUM MINERALIZATION, TEMPORARY</td><td>8</td><td>C-D</td></tr>
<tr><td>20</td><td>ISB, HIGH OR VERY HIGH MINERALIZATION, PERMANENT</td><td>5</td><td>C</td></tr>
<tr><td>21</td><td>ISB, HIGH OR VERY HIGH MINERALIZATION, TEMPORARY</td><td>8</td><td>C-D</td></tr>
<tr><td>22</td><td>ISB, HYPERSALINE, PERMANENT</td><td>6</td><td rowspan="2">E</td></tr>
<tr><td>23</td><td>ISB, HYPERSALINE, TEMPORARY</td><td>9</td></tr>
<tr><td>24</td><td>ISB, FLUVIAL ORIGIN, FLOOD PLAIN, LOW OR MEDIUM MINERALIZATION</td><td rowspan="4">3</td><td>A-B</td></tr>
<tr><td>25</td><td>ISB, FLUVIAL ORIGIN, FLOODPLAIN, HIGH OR VERY HIGH MINERALIZATION</td><td>C</td></tr>
<tr><td>26</td><td>ISB, FLUVIAL ORIGIN, FLOODPLAIN, ABANDONED MEANDER</td><td rowspan="2">B</td></tr>
<tr><td>27</td><td>ISB, ASSOCIATED TO ALKALINE PEATS</td></tr>
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<tr><td>30</td><td>COASTAL AMONG DUNES, TEMPORARY</td><td>7</td><td></td></tr>
</table>

eas, open water, among vegetation) using two handheld nets with mesh sizes of 100 μm for cladocerans and copepods and 1 mm for large branchiopods. Specimens were preserved in 4% formaldehyde. In the laboratory, most taxa were identified to species.

RESULTS

Types of lakes in Mongolia

Five types of lakes can be defined in Mongolia according the following descriptors: morphometry, hydroperiod, mineralization, and inorganic turbidity. This approach is similar to that adopted to classify lakes and lagoons in Spain by Alonso (1998). A brief description of the five types is given below.

- Type A. Large permanent freshwater lakes. In this type of lake, the maximum depth exceeds that which might be colonized by submerged aquatic vegetation, so lake metabolism is controlled by the plankton (trophic status). The specific electrical conductance is below 4 000 μS/cm. The biota is freshwater stenohaline. Fish are present.

- Type B. Smaller permanent or semipermanent freshwater lakes. In these lakes, aquatic submerged vegetation can colonize almost all of the lake bottom and therefore controls lake metabolism. Water turbidity is not due to inorganic suspended particles. Specific electrical conductance is below 4 000 μS/cm. The biota is freshwater stenohaline. Fish are present in lakes deeper than 4 m (ice cover can exceed a thickness of 2 m in winter).

- Type C. Large lakes and lagoons, either permanent or temporary, with highly mineralized or even saline (not hypersaline) waters. Specific electrical conductance between 4 000 and 45 000 μS/cm. The biota is euryhaline. Fish are present.

- Type D. Shallow lakes and lagoons, either permanent or temporary, with slightly to highly mineralized waters, turbid due to suspended inorganic particles. Specific electrical conductance 4 000-42 500 μS/cm. Turbidity does not allow submerged macrophyte growth. Lake metabolism is heterotrophic. Euryhaline biota. No fish present.

- Type E. Hypersaline lakes. Specific electrical conductance from 45 000 to more than 200 000 μS/m. Stenohaline athalassic saline biota. No fish present.

Branchiopod and copepod assemblages in Mongolia

The inventory of the Mongolian microcrustaceans on which this paper is based includes eighty-seven branchiopod and forty-nine copepod species.

Branchiopods include 10 Anostraca, 4 Conchostraca, 2 Notostraca, 3 Ctenopoda, 1 Haplopoda and 66 Anomopoda species. With respect to the Iberian fauna (Alonso, 1996; 1998), anostracans, conchostracans and notostracans are the most distinct groups, since only two anostracan species coincide. Of the three ctenopods, two are known in the Iberian Peninsula. Although haplopods do not occur on the Iberian Peninsula, there are 49 anomopod species in common between the two areas.

The copepods include 20 Calanoida, 26 Cyclopoida and 3 Harpacticoida species. Of the calanoids, which are prone to geographical speciation, only three species occur in both areas, whereas twenty cyclopoid species are shared between Mongolia and the Iberian Peninsula. All harpacticoids found in Mongolia also live in the Iberian Peninsula.

DISCUSSION

The use of Mongolian lakes as reference conditions for the ABCO subindex relies on two assumptions: (1) Spanish and Mongolian lakes are typologically equivalent, and (2) microcrustacean assemblages in lakes of the same type resemble one another when undisturbed.

Table 2. Characteristic Branchiopoda and Copepoda assemblages in the different types of lakes in Spain and in Mongolia. Geographically vicarious taxa in bold. *Asociaciones características de Branchiopoda y Copepoda en los diferentes tipos de lagos en España y en Mongolia. En negrita los taxones con vicarianza geográfica.*

Type of lake	**A**	**B**	**C**	**D**	**E**
Characteristic alliance (Alonso 1998)	Daphnion longispinae	Simocephalion vetuli	Arctodiaptomion	Mixodiaptomion incrassati	Artemion Arctodiaptomion
Characteristic associations (Alonso 1998)	*Chydoro-Eucyclopidetum serrulati,*	*Eurycercetum lamellati,*	*Simocephalo-Daphnietum magnae*	*Mixodiaptometum incrassati*	Association of *Artemia parthenogenetica*
	Acanthocyclopidetum	*Simocephaletum vetuli*	*Arctodiaptometum wierzjeski*	*Triopsetum mauritanici*	*Arctodiaptometum salini*
		Hemidiaptomo-Chirocephaletum diaphani	*Alona salina*	*Branchipetum schaefferi*	
Branchinecto-Daphnietum atkinsoni					
Characteristic species in Spain	*Alona affinis*	***Chirocephalus diaphanus***	*Daphnia magna*	***Branchipus*** sp. pl.	***Artemia parthenogenetica***
	Alona guttata	*Simocephalus vetulus*	*Simocephalus exspinosus*	***Branchinecta ferox***	***Phallocryptus spinosa***
	Coronatella rectangula	*Scapholeberis rammneri*	*Pleuroxus letourneuxi*	***Chirocephalus diaphanus***	*Branchinectella media*
	Chydorus sphaericus	*Eurycercus lamellatus*	***Alona salina***	*Streptocephalus torvicornis*	*Daphnia mediterranea*
	Eucyclops serrulatus	*Chydorus sphaericus*	***Arctodiaptomus wierzjeskii***	***Cyzicus grubei***	*Moina salina*
	Macrocyclops albidus	***Pleuroxus aduncus***	*Diacyclops bicuspidatus*	***Triops cancriformis mauritanicus***	*Arctodiaptomus salinus*
		Coronatella rectangula	*Megacyclops viridis*	*Daphnia atkinsoni*	*Cletocamptus retrogressus*
		Graptoleberis testudinaria		*Daphnia similis*	
		Hemidiaptomus roubaui		*Moina brachiata*	
		Diaptomus cyaneus		*Pleuroxus letourneuxi*	
		Eucyclops serrulatus		*Mixodiaptomus incrassatus*	
Characteristic species in Mongolia	*Alona affinis*	***Chirocephalus bobrinskii***	*Daphnia magna*	***Branchipodopsis affinis***	***Artemia sinica***
	Alona guttata	*Simocephalus vetulus*	*Moina mongolica*	***Branchinecta orientalis***	*Branchinectella media*
	Alona werestschagini	*Eurycercus lamellatus*	***Alona flossneri***	***Phallocryptus tserensodnomi***	*Moina salina*
	Coronatella rectangula	*Chydorus sphaericus*	***Arctodiaptomus salinus***	***Galaziella*** sp. pl.	*Metadiaptomus asiaticus*
	Chydorus sphaericus	***Pleuroxus truncatus***	*Megacyclops viridis*	***Cyzicus davidi***	*Cletocamptus* cf. *retrogressus*
	Eucyclops dumonti	*Pleuroxus annandalei*		***Triops*** cf. ***granarius***	
	Macrocyclops albidus	***Hemidiaptomus ignatovi***		*Daphnia similis*	
		Arctodiaptomus acutilobatus		*Moina brachiata*	
				Arctodiaptomus rectispinosus	
				Metadiaptomus asiaticus	
				Metacyclops minutus	

Table 1 shows the relationship between the lake typologies of the IPH and IBCAEL for Spain and those categories defined for Mongolia. The IPH establishes thirty types of lakes in Spain. Lake typology was defined according to the origins of the lake basins (glacial, karstic, fluvial, coastal), altitude, mineralization and type of hydroperiod. The IPH also considers as a distinguishing feature the location in interior sedimentation basins, which is clearly related to steppe conditions in Spain (Suarez Cardona *et. al*., 1992).

The IBCAEL typology distinguishes nine types. Except for types 6, 8 and 9, which univocally correspond to the IPH types 22, 19 and 23, the rest correspond to groups of IPH types having similar characteristic microcrustacean assemblages with regard to the ABCO subindex.

Excluding IPH types 28, 29 and 30, which are coastal, the rest of the Spanish lake types have a homologous type in Mongolia. Mountain and large karstic lakes in Spain (IPH types 1-12 and 14) correspond mostly to IBCAEL typologies 1, 2, and type “A” in Mongolia. IPH types 13, 15, 16, 17, 24, 26 and 27, which are generally freshwater shallow lagoons, can be assimilated to type “B” in Mongolia or to type “D” if the water is turbid because of inorganic particles. IPH types 18-20 and 25, which are characterized by medium or high mineralization, can be assimilated to type “C” in Mongolia or also “D” if they have inorganic turbidity. In addition, the IPH types that are hypersaline can be assimilated to type “E” in Mongolia.

The comparison of the benthic microcrustacean assemblages between the types of lakes and lagoons in Mongolia and in Spain is shown in Table 2. In Alonso (1998), the Spanish microcrustacean communities were characterized by studying the similarity among 470 inventories from a group of Spanish lakes and lagoons representative of all existing types and all regions in the country. The analysis distinguished five taxocenoses, which were designated alliances and associations and helped to finally define the typology of lakes and lagoons in Spain. This typology coincides with that established in Mongolia because it is based on the same principles. “Characteristic species” in Table 2 indicates the most frequent taxa in each taxocenosis so that the absence of one taxon in one of the assemblages does not necessarily imply that it is absolutely absent in the country to which the assemblage in question applies.

For type “A”, most taxa are coincident. For the other types, the taxa that could be used in Mongolia to establish reference conditions are mainly geographically vicarious, coinciding in most cases at the genus level. Some cases are interesting, such as *Arctodiaptomus salinus*, which has been found in Spain in El Tobar (Miracle *et al*., 1993) and Banyoles karstic lakes (Miracle, 1976) but also in most hypersaline endorheic lakes (Alonso, 1990); such ambivalence has never been investigated, and there are likely two different species under this specific name. In Mongolia, *A. salinus* never appears in hypersaline waters, only in highly mineralized waters, being *Metadiaptomus asiaticus* which characteristically inhabit hypersaline waters..

According to the observations presented here, the use of Mongolian lakes as reference conditions for the IBCAEL index in Spain, mainly for steppe lakes, seems to be realistic. The next step should include the necessary statistical analysis to assign to each taxon the sensibility value which, as was done in Spain, is based on an analysis of the fidelity of the different taxa to each determined lake typology.

ACKNOWLEDGEMENTS

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Does the nutrient concentration of water ecosystems affect growth rates and maximum PSII quantum yield in calcium alginate-encapsulated *Scenedesmus ovalternus* and *Chlorella vulgaris*?

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ABSTRACT

Does the nutrient concentration of water ecosystems affect growth rates and maximum PSII quantum yield in calcium alginate-encapsulated *Scenedesmus ovalternus* and *Chlorella vulgaris*?

Aquatic ecosystems are susceptible to deterioration caused by eutrophication. Changes in the nutrient concentration may affect species physiology, making it a key factor in structuring communities. Phytoplankters have a short generation time and a fast response to environmental factors, which makes them a good model to address issues related to the effects of the trophic status on aquatic organisms. Our aim was to determine the changes in the growth and maximum PSII quantum yield of calcium alginate-encapsulated *Scenedesmus ovalternus* and *Chlorella vulgaris* incubated in aquatic ecosystems with different nutrient concentrations. We tested the following hypotheses: 1) alga with a greater capacity for nutrient absorption (*C. vulgaris*) would have the highest growth regardless of the environment nutrient concentration and 2) the concentration of nutrients positively affects the maximum PSII quantum yield in the two species. To test the hypotheses, *S. ovalternus* and *C. vulgaris* were immobilized in calcium alginate and cultured in wetlands with different nutrient concentrations. The growth of the two species differed between the wetlands; higher development was observed in the eutrophic environment. Significant differences were only recorded between the species in the eutrophic system, with greater growth in *C. vulgaris*. The hypereutrophic environment conditions were lethal for both species. The maximum quantum yield of PSII showed similar behaviour in both optimum nutrient conditions and limiting conditions. The nutrient concentration of the studied environments influenced the growth of the two species but not their maximum quantum yield of PSII, which seemed to be affected by factors other than nitrogen (N) and phosphorus (P) concentrations. *C. vulgaris* presented optimum growth only in the eutrophic system. The results showed that the growth rates of encapsulated algae could be a useful method for assessing changes, such as nutrient concentration, in the environmental conditions of the Sabana de Bogotá wetlands.

Key words: Algae immobilization, tropical high mountain wetland, biological monitoring, phytoplankton dynamic.

RESUMEN

¿La concentración de nutrientes de los ecosistemas acuáticos afecta la tasa de crecimiento y la eficiencia cuántica máxima del fotosistema II de* Scenedesmus ovalternus *y* Chlorella vulgaris *encapsuladas en alginato de calcio?

Los ecosistemas acuáticos son susceptibles al deterioro causado por los procesos de eutrofización. Los cambios en la concentración de nutrientes pueden afectar la fisiología de las especies y de esa forma ser un factor clave en la estructuración de las comunidades. Los organismos fitoplanctónicos presentan tiempos de generación cortos y respuestas rápidas a los factores ambientales, lo que los hace un modelo apropiado para abordar temas relacionados con el efecto del estado trófico sobre organismos acuáticos. Nuestro objetivo en este trabajo fue determinar los cambios en el crecimiento y en la eficiencia cuántica máxima del fotosistema II de Scenedesmus ovalternus *y* Chlorella vulgaris *encapsuladas en alginato de calcio e incubadas en ecosistemas acuáticos con diferente concentración de nutrientes. Las hipótesis a probar fueron 1) que el alga* *con una mayor capacidad de absorción de nutrientes (*C. vulgaris*) presenta el crecimiento más elevado sin importar la concentración de nutrientes del ambiente; y 2) que la concentración de nutrientes afecta de forma positiva la eficiencia cuántica máxima del PSII de las dos especies. Para probar estas hipótesis,* S. ovalternus *y* C. vulgaris *fueron inmovilizadas en alginato de calcio y expuestas a cuatro humedales de concentración de nutrientes. El crecimiento de las dos especies difirió entre humedales, con mayor crecimiento en el sistema eutrófico. Solo en ese sistema se registraron diferencias significativas entre las especies, con mayor crecimiento para* C. vulgaris*. Las condiciones del ambiente hipereutrófico (excesivamente rico en nutrientes) fueron letales para las dos especies. La eficiencia cuántica máxima del fotosistema II mostró un comportamiento similar tanto en situaciones óptimas de nutrientes como en condiciones limitantes. La concentración de nutrientes de los ambientes estudiados influenció el crecimiento de las dos especies pero no su eficiencia cuántica máxima del fotosistema II, que parece estar determinada por otros factores además de las cantidades de nitrógeno y fósforo.* C. vulgaris *tuvo un crecimiento óptimo solo en el sistema eutrófico. Los resultados mostraron que las tasas de crecimiento de las algas encapsuladas podrían ser un método útil para valorar los cambios en las condiciones ambientales de los humedales de la Sabana de Bogotá, como es el caso de la concentración de nutrientes.*

Palabras clave: *Inmovilización de algas, humedales tropicales de alta montaña, monitoreo biológico, dinámica del fitoplancton.*

INTRODUCTION

Inland aquatic ecosystems are susceptible to deterioration due to eutrophication processes that result from human activities. In aquatic environments, changes in nutrient concentrations can affect the structure of biological communities and ecosystems (Kulikova & Syarki, 2004; Ristau *et al*., 2012; Da Silva *et al*., 2014; Snickars *et al*., 2014). For phytoplankters, an increase in nutrient availability promotes the spread of some species but inhibits others (Tilman *et al*., 1982; Caputo *et al*., 2008; Zhu *et al*., 2010). The presence and abundance of particular phytoplankton species have been associated with the quantity of nutrients (Reynolds, 2006; Bellinger & Sigee, 2011). The abundance of a taxon in an ecosystem depends on its morphological and physiological characteristics (Reynolds, 2006), the availability of nutrients and light, the rate of nutrient uptake and the rate of biomass loss (i.e., sedimentation, washing, physiological death, herbivory, Tilman *et al*., 1982). The classical theory of succession determined by nutrients postulates that the growth of some microalgae species will be higher at low nutrient concentrations, while other taxa will present greater growth when the nutrient concentration is high (Margalef, 1978; Sommer, 1989; Reynolds, 2006); thus, the distribution of species and their abundance vary with the trophic gradient (Reynolds, 1998).

To determine the trophic characteristics of an aquatic environment based solely on nutrients can be difficult, especially when nutrients are limiting (Hudson *et al*., 2000; Rattan *et al*., 2012). Alternatively, it is possible to evaluate the direction of trophic changes in lakes indirectly via the physiological responses of autotrophic microorganisms, e.g., biomass production (Jaworska & Zdanowski, 2012) and growth rate (Chrzanowski & Grover, 2001); moreover, nutrient stress can be tested utilizing the maximum photochemical efficiency of photosystem II (F_v/F_m) (Parkhill *et al*., 2001), where F_v is the variable fluorescence of chlorophyll a (the difference between maximum and minimum fluorescence), and F_m is the maximum fluorescence. F_v/F_m is a measurement routinely used to determine the effects of environmental factors (e.g., nutrients and temperature) on phytoplankton photosynthetic efficiency (Cleveland & Perry, 1987; Kolber *et al*., 1988; Cullen *et al*., 1992; MacIntyre *et al*., 1997; Parkhill *et al*., 2001; Rattan *et al*., 2012; Wang *et al*., 2014). Laboratory studies have associated high and constant values of F_v/F_m to nutrient saturation conditions, while low values of F_v/F_m are linked to limiting conditions (Geider *et al*., 1993; Wang *et al*., 2014). *In situ* F_v/F_m readings reflect the total response of the phytoplankton community, and it is difficult to interpret the effects of nutrient concentration on the photosynthetic efficiency of individual species (Rattan *et al*., 2012).

In this study, we used two microalgae species with different morphological and metabolic characteristics: *Chlorella vulgaris* (Beijerinck, 1890) and *Scenedesmus ovalternus* (Chodat, 1926). Because of its small size and rapid growth, the unicellular algae *C. vulgaris* can be characterized as an r-strategist (Pianka, 1970) with a high surface-volume ratio and is functionally classified in group I of the morpho-functional categorization proposed by Kruk *et al*. (2010). According to Reynolds (2006), *C. vulgaris* belongs to a group of algae that are characterized as organisms that have low biomass loss through sedimentation and have adapted to rapid nutrient acquisition. Organisms of this type are sensitive to nutrient deficiencies and are usually present in well-mixed shallow eutrophic lentic environments (Reynolds *et al*., 2002). On the other hand, the cenobial algae *Scenedesmus ovalternus* is associated with eutrophic to hypereutrophic shallow lakes and rivers (Bellinger & Sigee, 2011). It is sensitive to low light intensity (Reynolds, 2006) and is functionally classified as a medium-sized species with a moderate tolerance to low nutrient concentrations (Group IV, Kruk *et al.*, 2010). *S. ovalternus* has a lower speed nutrient acquisition and a higher rate of biomass loss through sedimentation than *C. vulgaris* (Reynolds, 2006).

Our aim was to determine the growth and F_v/F_m responses of two algae species with different functional characteristics embedded in alginate beads and cultured under waters with different concentrations of nutrients. To achieve our goal, the cultures of each species were immobilized in sodium alginate beads and cultured in water bodies at different trophic states (oligotrophic to hypereutrophic). Immobilization allowed the respiratory and photosynthetic activities of the algae to occur and avoids cell loss through herbivory and sedimentation (VanDonk *et al*., 1993; Faafeng *et al*, 1994). The alginate matrix does not affect light penetration and has very little effect on the self-diffusion of small molecules such as nutrients (Tanaka *et al*., 1984). However, alginate beads with high cellular density would reduce the amount of light diffusion, which would affect the metabolism of algal cells (Hameed, 2013). Regarding the effects of encapsulation on algae, studies with *Scenedesmus obliquus* (Chevalier & de la Noüe, 1985) and *Chlorella vulgaris* (Lau *et al.*, 1998) have shown that the lag phase is longer for immobilized algae but that the maximum growth rate is similar for encapsulated and free algal cells. In addition, some studies have revealed that immobilization has little effect on the morphology of colonial, filamentous and unicellular algae taxa (Musgrave *et al.*, 1983; Bailliez *et al*., 1985; Trevan & Mak, 1988).

We tested the following hypotheses: 1) the algae species with the greatest capacity to absorb nutrients (*C. vulgaris*) will exhibit the highest population growth regardless of the water nutrient content and 2) the concentration of nutrients will positively affect F_v/F_m in both unicellular and coenobium species, meaning that algae in the system with the highest concentration of nutrients will present higher F_v/F_m values.

MATERIALS AND METHODS

Study sites and limnological characterization

We conducted our experiments during the dry (February-March) and rainy (April-June) seasons of 2013. We selected three aquatic lentic ecosystems with different trophic statuses in the Bogotá River basin (between 2560 and 2900 masl), which was trophically characterized by Rodríguez (2012): the San Rafael reservoir (lower trophic level or oligo-mesotrophic, 4°42′10.6″N 73°59′26.4″W); the Santa María wetland (moderate trophic level or mesotrophic, 4°41′40.3″N 74°05′34.0″W); and the La Gaitana zone at the Juan Amarillo wetland (hypereutrophic, 4°44′13.1″N 74°06′47.5″W). To increase the environmental variability in our assays, during the rainy season, we included one more ecosystem with eutrophic status (La Conejera wetland, 4°45′42.8″N 74°06′13.7″W) (Acosta & Chivatá, 2016). A detailed description of the physical and morphological characteristics of these wet-

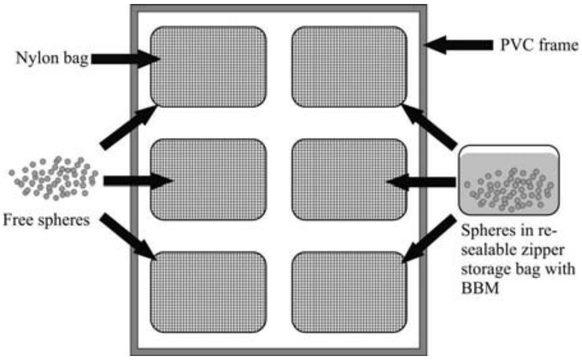

Figure 1. Diagram of the PVC structure for the experiments with immobilized algae carried out in aquatic ecosystems of the Sabana de Bogotá. *Esquema de la estructura en PVC para los experimentos con algas inmovilizadas llevados a cabo en los ecosistemas acuáticos de la Sabana de Bogotá.*

lands can be found in the studies of Consevación Internacional Colombia (2003) and Rodriguez (2012).

In each season and aquatic ecosystem, we measured the following environmental variables for ten days: photosynthetically active radiation (subsurface and at a 10 cm depth-PAR, μmol s^{-1} m^{-2}), electrical conductivity (Cond, μS/cm), dissolved solids (DS, mg/l), pH (units), oxygen (O_2, mg/l and saturation percentage, O_2%), temperature (°C), redox potential (RP, mV), and chlorophyll-*a* (mg/l). Additionally, on the first, fifth and tenth days of the experiments, we determined the concentration of ammonia (mg/l), total nitrogen (mg/l), total phosphorus (mg/l) and the chemical oxygen demand (COD, mg/l). All of these parameters were measured on the littoral of the ecosystems, where the encapsulated algae incubations were also made. For all procedures, the methodologies of APHA *et al*. (1995) were followed. We calculated the water light transmittance using the PAR measurements (Kirk, 2011) as follows:

$$Tr = \frac{l_z}{l_0} \times 100,$$

where l_z is the irradiance at z depth, and l_0 is the irradiance just below the water surface.

A principal component analysis (PCA) was performed to summarize the environmental variables (Legendre & Legendre, 1998) and determine the similarities of the environmental conditions between waterbodies and seasons. The variables were previously log-transformed (except pH) and standardized. The axis retention was evaluated using the Broken-Stick criterion (Jackson, 1993). For each season, the variation of the more important environmental variables evidenced in the PCA was tested using one-way analysis of variance (ANOVA), with waterbody as the factor.

We established the atomic N:P ratio and the trophic state of each environment using trophic indices for nitrogen (TSI_{TN}) (Kratzer & Brezonik, 1981), phosphorus (TSI_{TP}), and chlorophyll-*a* (TSI_{Chl}) (Carlson, 1977). The mean trophic state (TSI_{mean}) was calculated as follows:

$$\frac{[TSI_{TN} + TSI_{TP} + TSI_{Chl}]}{3}.$$

Preparation of immobilized algae beads

We used the species *Scenedesmus ovalternus* (LAUN 001 strain) and *Chlorella vulgaris* (LAUN 002 strain), which were both supplied by the Algal Culture Laboratory of the Departamento de Biología at the Universidad Nacional de Colombia. A solid inoculum of each species was added to 375 ml culture flasks with Bold Basic Medium (BBM) at a constant temperature (23 °C ± 2 °C). A light intensity of 60 μEm^{-2} s^{-1} was applied with a 16:8 h light-dark cycle. Cultures were grown to the stationary growth phase.

Subsequently, an aliquot of the culture was taken for each species, which was mixed with a 4% solution of sodium alginate at a 1:1 volume ratio in order to obtain a 2% solution of algae-alginate (the final cellular concentration is shown in Table 1). This new solution was placed in 5 ml syringes and allowed to drip into a solution of calcium chloride (4%). With this procedure, approximately 1200 spheres (diameter ≈ 3 mm) were produced per 50 ml of 2% algae-alginate solution. The beads were kept in 2% $CaCl_2$ at 6 °C for 24 hours to ensure hardening. This immobilization procedure has been used elsewhere on these algae genera with great results (Mallick, 2002).